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Article

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**Using variable ionisation energy time-of-flight
mass spectrometry with comprehensive
GC×GC to identify isomeric species**

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ABSTRACT

Although GC×GC-ToF-MS allows the separation of thousands of peaks, many of these peaks are not positively identified owing to the lack of mass spectral library data and/or standard materials, leading to a substantial amount of information being inaccessible. The fragmentation patterns of molecules in mass spectrometers using electron impact ionisation at 70 eV can be useful for molecule identification, provided a match is available in a published EI MS library, but are indistinguishable for many isomeric organic compounds (for example, aliphatic and branched alkanes). Lower ionisation energies have been exploited leading to organic compounds being ionised with lower excess internal energy and less fragmentation, retaining the molecular ion and maximising its relative signal. This has enabled the identification of a large number of isomeric organic compounds, both aliphatic and aromatic, between C₁₂-C₃₆, in the previously unresolved complex mixture (UCM) of two motor oil samples.

This technique also demonstrates problems associated with separation of co-eluting isomers, particularly for the *n*-alkanes, which are routinely measured by 1D GC/MS and may be overestimated, due to co-elution. As a consequence retention times in 2 dimensions and mass spectra at variable ionisation energies are shown to give unparalleled power to identify specific isomers.

Keywords: GC×GC, gas chromatography, mass spectrometry, ionisation, *n*-alkanes, fragmentation

INTRODUCTION

There is a well-established need for the characterisation of organic matter in a range of media. The study of organic compounds in the atmosphere has been an interest of scientists for many years owing to the potential detrimental health effects.¹⁻⁴ The complexity of the atmosphere as well as the drawbacks associated with current analytical techniques have limited studies in investigating specific compound classes and/or target analytes. Thus, detailed knowledge of the identities and chemistry of organic compounds as a whole remains elusive. Goldstein and Galbally⁵ illustrated the exponential increase of number of unique isomers possible as a function of the number of carbon atoms in an organic molecule, with *ca.* 1×10^6 constitutional isomers possible for a C₂₀ alkane.

The study of organic compounds within crude oils has also generated much interest as it plays an important role in petroleum exploration. Crude oils have a limited number of compound classes, but an enormous number of individual components within a class. The composition of crude oil is also susceptible to physical, chemical, biological and weathering processes, leading to significant differences between any two crude oil samples.⁶ Sutton et al.⁷ isolated individual hydrocarbon fractions from an unresolved complex mixture (UCM) of hydrocarbons isolated from a biodegraded crude oil and suggested that the entire oil UCM may contain *ca.* 250,000 unidentified compounds. This suggests that a substantial fraction of chemical composition information is inaccessible and therefore unexploited.

The most common method for the speciation of organic compounds has been gas chromatography–mass spectrometry (GC/MS). This technique has been exploited to separate crude oils;⁷⁻⁹ airborne samples which encompass multiple emission sources containing multiple compound classes and both polar and non-polar components;¹⁰⁻¹³ and sediments which can contain fingerprints of both crude oils and various other source emissions.^{6,14} The peak capacity of conventional GC, however, is insufficient to resolve the full complexity of these mixtures and a pronounced rising baseline or

series of rising baselines in the gas chromatogram are observed, often referred to as the unresolved complex mixture (UCM).¹⁵ For example, the UCM in conventional GC separation of oil-based samples typically accounts for *ca.* 95 % of the area of the chromatogram and is usually unresolved by analysis. It is thought to comprise a major fraction of constitutional linear, branched and cyclic alkane isomers and a minor fraction of aromatic compounds.^{16,17} Due to the difficulties in identifying isomers, information regarding their molecular structures has generally been unavailable. This knowledge, however, is crucial in understanding the environmental fates of these compounds, as the degree and positioning of branching in these alkanes strongly affect their oxidation mechanisms in the environment and secondary organic aerosol (SOA) formation yields in the atmosphere.¹⁸⁻²⁰

Multidimensional or comprehensive gas chromatography (GC×GC) provides enhanced peak capacity and separating powers, due to an orthogonal separation. This technique utilises two capillary columns of different stationary phases connected in series by a modulator, which delivers discrete bundles of effluent from the primary column to the secondary column.^{21,22} Compounds of similar chemical structure are grouped into distinct patterns in ordered chromatograms, providing useful information that aids identification.²³ GC×GC is often coupled with time-of-flight mass spectrometry (ToF-MS) which provides extra information in the identification of compounds, due to its fast data acquisition rate, full range mass sensitivity and, in some cases, high resolution. These advantages make GC×GC-ToF-MS an attractive analytical technique for separating complex mixtures, measuring a wide range of volatile and semi-volatile organic compounds in air and oils.²⁴⁻

²⁷

Although GC×GC-ToF-MS allows the separation of thousands of peaks, many of these peaks are not positively identified owing to the lack of mass spectral library data and/or standard materials.²⁸ This means that a substantial amount of information is still inaccessible. Traditional mass

spectrometers (MS) employ electron impact (EI) ionisation at 70 eV, where a molecule undergoes ionisation by an electron (most organic molecules have ionisation energies <11 eV). The electron imparts a large amount of excess energy causing extensive fragmentation. The fragmentation mass spectra can in most cases be useful for molecule identification, provided a match is available in a published EI MS library, but for many organic compounds, such as the isomeric alkane structures found in the UCM, are indistinguishable. This is because the fragment ions found in the mass spectra of aliphatic compounds, for example alkanes, are very similar *i.e.* m/z 43, 57, 71, 85, 99 *etc* and the molecular ion is often barely visible or absent. Interpretation of EI-MS spectra of complex mixtures can therefore be ambiguous. These limitations in EI have led to a continual demand for more robust lower (or soft) ionisation techniques for MS. Using lower ionisation energies can lead to organic compounds being ionised with minimal excess internal energy and thus less fragmentation, hence retaining the identity of the molecule by retaining a much larger fraction of the molecular ion. This has been the subject of rigorous research by Maccoll and co-workers,²⁹ since their early work on ion enthalpies and their application in mass spectrometry.³⁰ A series of literature has since been published, in seventeen parts over a decade, reporting low EI (12.1 eV), low temperature (~350K) mass spectra of various compound classes.

Several soft ionisation techniques have been developed such as chemical ionisation (CI),^{31,32} field ionisation (FI)^{33,34} and photoionisation (PI).^{35,36} CI typically utilises reagent ions produced by EI of a reagent gas (such as methane, isobutane or ammonia) at high pressure (*ca.* 1×10^{-4} mbar). The reagent ions are only slightly reactive with the reagent gas itself, but readily react to ionise the sample via ion-molecule reactions. Although this ionisation technique often gives molecular mass information with reduced fragmentation compared to EI, results are highly dependent upon reagent gas type, pressure, reaction time and the nature of the sample. In addition, CI requires a different ion-source configuration and thus it can be time consuming and resource intensive if a single MS instrument is being used for both EI and CI. FI has been exploited by a number of studies.³⁷⁻³⁹ FI

125 produces dominant molecular ions with little or no fragmentation. Hejazi et al.³⁸ developed a
126 method that utilises GC with parallel hard (EI) and soft ionisation (FI) mass spectrometry. This
127 instrument consisted of two mass spectrometers, with differing ionisation methods, connected to a
128 single GC. Wang et al.³⁹ investigated the separation of diesel fuel using a two dimensional
129 separation approach whereby FI-MS was coupled to a GC. FI, however, produces weak ion currents
130 with significantly more fluctuation on the one second time scale of a typical scan for a scanning
131 MS, resulting in poor repeatability of the relative abundances of measured ions.³⁷

132
133 An increasingly popular soft ionisation technique is PI, where principally two different PI
134 techniques have been investigated, namely resonance-enhanced multi-photon ionisation (REMPI)
135 and single photon ionisation (SPI).³⁵ REMPI utilises intense ultraviolet (UV) light laser pulses for a
136 two photon absorption process, whereas SPI uses vacuum ultraviolet (VUV) photons for a single
137 photon absorption process. REMPI is highly sensitive and selective for specific compounds such as
138 aromatics and polyaromatic compounds,^{36,40,41} which prevents this soft ionisation technique from
139 being applicable to crude oil samples that are rich in aliphatic compounds. SPI, however, with VUV
140 is capable of soft ionisation of all organic compound classes including the aliphatic alkanes.^{35,42}
141 Isaacman et al.⁴³ used VUV ionisation at 10 – 10.5 eV to elucidate some of the structures within the
142 UCM of atmospheric particles, by separating components using GC. Chan et al.⁴⁴ also exploited
143 this technique in order to characterise the UCM and to provide insights into atmospheric processing
144 of these components. Their results showed that the most abundant isomer for each carbon number
145 was the linear alkane accounting for >30% of the total alkane mass. Drawbacks of SPI VUV,
146 however, are the relative experimental complexity of the technique and the reduced stability of the
147 molecular ions that are formed (radical cations) in comparison to other techniques such as MALDI
148 and atmospheric pressure CI (even-electron ions). It is therefore, important to develop and
149 investigate reliable, inexpensive and simple ionisation techniques that can help identify the highly
150 numerous unknown compounds that are present in samples such as aerosols and petroleum samples.

In this study, we use variable ionisation time-of-flight mass spectrometry, ranging from 10 to 70 eV coupled to GC×GC, to determine the structures of aliphatic and aromatic compounds within the UCM. By using soft EI and conventional 70 eV EI, both molecular ions and fragment ions can be obtained. EI provides high ion yield and sensitivity, generating highly reproducible spectra, for which there is a large database of spectra of known compounds (NIST). Soft ionisation provides the molecular ion and reduced fragmentation, for which there is no database available. Both mass spectral data at 14 and 70 eV, as well as retention times from the GC×GC, give insights into structural positioning and extent of alkyl branching of aliphatic (linear) alkanes and aromatics.

EXPERIMENTAL

Instrumentation

Sample analyses were conducted by a gas chromatograph (7890B, Agilent Technologies, Wilmington, DE, USA) equipped with a Zoex ZX2 cryogenic modulator (Houston, TX, USA). The first dimension was equipped with a SGE DBX5, non-polar capillary column (30 m, 0.25 mm ID, 0.25 μm – 5% phenyl polysilphenylene-siloxane), and the second dimension column was SGE DBX50 (4.0 m, 0.1 mm ID, 0.1 μm – 50% phenyl polysilphenylene-siloxane). The GC×GC was interfaced with a BenchTOF-Select, time-of-flight mass spectrometer (Markes International, Llantrisant, UK). The acquisition speed was 50 Hz with a mass resolution of >1200 FWHM at 70 eV and >800 FWHM at 14eV over 100 – 1000 m/z .⁴⁵ The mass range was 35 to 600 m/z . The GC sensitivity at 70 eV has been calculated by the injection of 1 pg octafluoronaphthalene (m/z 272) giving a signal-to-noise ratio (S/N) of >2000:1 rms, while for 14eV, the injection of 1 pg of benzophenone (m/z 182) gave a S/N of >80:1 rms.⁴⁵ Although ionisation energies can be varied from anywhere between 10 and 70 eV, explored energies in this work were 14 eV and 70 eV, by means of repeat injection runs. All data produced were processed using GC Image v2.3 (Zoex Corporation, Houston, US).

Principle of soft ionisation technique

For the conventional EI ion source, electrons are accelerated from the surface of a negatively charged filament towards a positively charged ion chamber, owing to a potential difference of 70 V, thus efficiently ionising compounds from the GC. Reducing this potential difference (for soft ionisation) results in a dramatic loss of sensitivity due to the inefficiency of drawing electrons into the ion chamber and clustering of electrons around the filament. The variable ionisation source uses ion optics in order to retain the high potential difference, thus accelerating the electrons from the filament and reducing their energy prior to arriving at the ion chamber.⁴⁶ This allows the ionisation energy to be tuned between 10 and 70 eV. Unlike CI and FI, no reagent gases, adjustments in pressure or switching of sources are required.

Methodology

Two motor/lubricating oil samples (synthetic 5W30 and pure base oil analogous to mineral oil, see Supporting Information for more details) were analysed, utilising GC×GC-ToF-MS to identify aliphatic isomeric compounds. The protocol applied to the motor oil analytical sample was to inject 1 µL of diluted (1:1000) sample in a split ratio 100:1 at 300 °C. The initial temperature of the primary oven (175°C) was held for 5 min and then increased at 1°C/min to 325°C. The secondary oven temperature program was the same as the primary oven but was increased at 1°C/min to 330°C to ensure all species passed through the column. The transfer line temperature was 325°C and the ion source temperature was 280°C. Helium was used as the carrier gas at a constant flow rate of 0.8 mL/min. A modulation time of 7 seconds was used, while a total run time of each sample was 120 minutes.

RESULTS & DISCUSSION

A typical two-dimensional separation is presented in Figure 1(A) and (B) for the base lubricating oil. The white line in Figure 1(A) illustrates the separation achievable using traditional 1D GC/MS.

Each coloured spot represents an individual compound with a corresponding full mass spectrum. The intense dark blue spots are major peaks, while the lighter blue spots represent small peaks. Compounds were separated in the first dimension (X-axis) on a volatility basis and polarity in the second (Y-axis).

Samples were run using two ionisation energies, 70eV and 14eV to gain insight on fragmentation patterns and molecular ions, respectively. The procedure for peak identification is outlined in detail elsewhere.²³ Briefly, peaks were identified based primarily on mass spectral data from the NIST library (for 70eV), where two match factors (MF>750 and RMF>800) were used to assess the similarity between the library and measured mass spectra. For samples analysed with lower ionisation energy (14eV), the identification of species was determined by interpretation of mass spectral data and comparing their retention indices and mass spectra at 70eV, together with the presence of the species molecular ion (possessing higher relative intensity than in a 70eV mass spectrum). The samples analysed at both ionisation energies therefore provided greater insight into a compound's identity, indicating the power of soft EI. Many differences were observed between the two oils (total ion chromatogram for 5W30 oil not shown in Figure 1A), the most notable being the presence of more high molecular weight (HMW) (less volatile) compounds in the 5W30 synthetic oil.

Monomethylalkanes

Monomethylalkanes are present in many natural and synthetic organic materials, including crude oil, coal and sediments;⁴⁷⁻⁵⁰ and have been proposed as endogenous biomarkers in exhaled breath.⁵¹ The separation and identification of monomethylalkanes in a broad range of carbon atoms is difficult owing to the number of isomeric possibilities and close retention times. Recently, Szabó et al.⁵² utilised GC×GC-FID and GC/MS/MS with 100m columns to separate 63 C₉-C₁₉ monomethylalkanes, reporting up to 8 isomers for the monomethyl-nonadecane (C₁₉) in exhaled

breath. Krkošová et al.⁴⁷ reported the linear retention indices of 196 C₄-C₃₀ monomethylalkanes using GC/MS equipped with a 100m column and used these retention indices to identify these species in diesel fuel. All these studies have considered a targeted approach in their analyses and not considered the presence of other compounds in complex mixtures.

The use of variable ionisation energy coupled to mass spectrometry, as well as retention time information enables a non-targeted approach to be conducted. For example, 2-methyl-eicosane and 3-methyl-eicosane are C₂₁H₄₄ isomers which have different retention indices and are largely experimentally indistinguishable from other aliphatic isomers using their fragmentation mass spectra at 70eV (see Figure 2A and 2D). The absence of the molecular ion (*m/z* 296) means that it is difficult to deduce, without retention time information, which isomeric aliphatic (with corresponding C number) it belongs to; as all aliphatic compounds with \geq C₂₂ show relatively small peaks at *m/z* 253 and *m/z* 267. At 14eV however, both these compounds are distinguishable, allowing the identification of the positioning of branching on an aliphatic alkane (see Figure 2C and 2F). The presence of the molecular ion at *m/z* 296 allows the identification of these compounds without the use of retention indices. The 14eV mass spectrum of 2-methyl-eicosane shows a relatively large peak at *m/z* 253, signifying a loss of a C₃H₇ fragment (see Figure 2C), while for 3-methyl-eicosane the presence of a significant peak at *m/z* 267 signifies the loss of C₂H₅ indicating the most likely point of cleavage (see Figure 2F). This information together with their identical retention indices in both 14eV chromatography and 70eV chromatography allows the identification of 7 further C₂₁ monomethylalkane isomers. For example, the 14eV mass spectrum of 4-methyl-eicosane showed the presence of peaks *m/z* 225 signifying the loss of a C₅H₁₁ fragment and *m/z* 253, signifying a loss of a C₃H₇ fragment (see Figure 2J). 2-Methyl and 4-methyl-eicosane mass spectra were distinguishable from one another, due to the presence of the *m/z* 225 fragment, together with their positioning in the chromatography which was confirmed using Kovats retention indices. The mass spectra of 5-methyl-eicosane shows relatively large peaks at *m/z* 239 and *m/z* 211 signifying

the loss of mass fragments C_4H_9 and C_6H_{13} , respectively (see Figure 2K). 6-Methyl-eicosane mass spectra exhibited peaks at m/z 225 and m/z 197, where the m/z 71 and m/z 99 peaks showed similar relative intensities (see Figure 2L). From these peaks together with the m/z 197, 6-methyl-eicosane was distinguishable from 4-methyl-eicosane (which has the same m/z 225 fragment). Peaks at m/z 225 and 197 corresponded to C_5H_{11} and C_7H_{15} fragment losses. This analogy was completed for 7, 8, 9 and 10-methyl-eicosanes; however, there is significant co-elution for 9-methyleicosane and 10-methyleicosane compounds (Figure 3(A)). All methyl-eicosane mass spectra retained the presence of the molecular ion at m/z 296 (see Figure 2A-2L).

As compounds with similar chemical structure are grouped into distinct patterns in ordered chromatograms, the order of branching with increasing retention time is consistent throughout the chromatogram with increasing carbon number (up to C_{28}). The faster eluting monomethylalkanes are those methylated alkanes with methyl groups furthest away from the first carbon position (with the exception of 2-methylmonoalkanes, determined by assessing each monomethylalkane's mass spectrum at 14eV and previously published literature.⁴⁷ Thus, for C_{21} isomers the order of branching with increasing retention time would be 10-, 9-, 8-, 7-, 6-, 5-, 4-, 2- and then 3-methyl-eicosane. The majority of these isomeric monomethylalkanes are present in the synthetic oil and are not all present within the base oil. For example, for C_{21} isomers, there is no 5-methyl-eicosane present in the base oil (see Figure 3A). Signal intensities of the monomethyl C_{24} – C_{28} alkanes in the base oil are also 20-30% lower than the synthetic oil, which may reflect the differences in the production and refining of the two oils (the base oil being an API group 1 oil and having ~77% saturates). These compounds were easily identifiable using 14eV spectra throughout the UCM for the base oil but not for the 5W30 oil, most probably due to the coelution of the larger number of isomers present in the latter. The base oil also consists of a number of di- tri- tetra- and penta-methylalkanes extending beyond each *n*-alkane species as shown in Figure 3(B), identified tentatively by their mass spectra at both ionisation energies (for $<C_{22}$) and published retention times (where available).

The tri- and tetra-methyl alkanes (belonging to the C_{23} carbon number) were not present in abundance in the 5W30 lubricating oil (see Figure 3) and elute faster than the C_{21} *n*-alkane. It must be noted that the first dimension is separated by volatility and is not a carbon number distribution. The mass spectra for many substituted methylalkanes with $>C_{22}$ were indistinguishable at 70 eV. Figure 4 illustrates the experimentally determined mass spectra for 2,6,10,14-tetramethyl-nonadecane ($C_{23}H_{48}$; upper panel) and 2,6,10,14,18-pentamethyl-nonadecane ($C_{24}H_{50}$; lower panel) at 70 eV (4A), 14 eV (4B) and their respective NIST reference spectra (4C). Although these compounds, in both motor oils, elute at their specific published retention times, they are indistinguishable at 70 eV. The mass spectral peaks are those found in all aliphatic compounds with several CH_2 (m/z 14) cleaved mass fragments. At 14 eV, however, the presence of the molecular ion and relatively larger intensities of ions that signify likelihood of the cleavage of a C-C bond, allows the positive identification of these compounds within complex mixtures. For example, for 2,6,10,14-tetramethyl-nonadecane, the loss of fragments $C_5H_{11} + C_6H_{13}$, $C_{10}H_{21} + C_{11}H_{23}$ and $C_{15}H_{31} + C_{16}H_{33}$ signify bond breaking either side of the methyl group, indicating the positioning of the methyl carbon bond and give rise to m/z 253+239, 183+169, and 113+99, respectively. While for 2,6,10,14,18-pentamethyl-nonadecane the loss of $C_{11}H_{23}$ and C_8H_{17} fragments results in relatively large peaks at m/z 183 and 113, respectively. This information as well as the presence of the molecular ion enables confident structural assignment of these compounds. It is interesting that the NIST (70eV) reference spectrum looks significantly softer than the experimental 70eV spectrum and remarkably similar to the 14eV ionisation mass spectrum. The NIST library at 70 eV also signifies the presence of the molecular ion (see Figure 4C); however, this is not easily observed for many aliphatics within complex mixtures, particularly for high molecular weights.⁵³

n-Alkanes

n-Alkanes are ubiquitous in the environment. They are biosynthesized by several organisms such as terrestrial and aquatic plants, algae and insects and are used as biomarkers in biogeochemistry.⁵⁴ *n*-

Alkanes are a major constituent of crude oil and are routinely and easily measured by GC/MS. In petroleum polluted sediments the *n*-alkanes generally range between C₁₂ and C₄₀, whereas in marine organisms the range is normally <C₂₁.⁵⁵

Diagnostic ratios such as, natural *n*-alkane ratio (NAR), carbon preference index (CPI), terrigenous/aquatic ratio (TAR) and low molecular weight (LMW)/HMW ratios are exploited to assess possible sources of *n*-alkanes. For example, NAR estimates the proportion of natural and petroleum *n*-alkanes, where ratios close to zero signify petroleum hydrocarbons and crude oil sources, while ratios close to one suggest terrestrial or marine plant sources.⁵⁶ CPI are ratios between odd and even numbered *n*-alkanes, where values in the range *n*-C₂₄ and *n*-C₃₂ close to one are found for crude oils and other petroleum sources, while a predominance of odd numbered *n*-alkanes is characteristic of terrestrial plant debris.^{55,57,58} TAR is used as a crude indicator of relative terrigenous versus aquatic organic matter input, where the ratio between the concentrations of long chain *n*-alkanes (*n*-C₂₇ + *n*-C₂₉ + *n*-C₃₁) and short chain *n*-alkanes (*n*-C₁₅ + *n*-C₁₇ + *n*-C₁₉) is evaluated.⁵⁹ All these ratios are crude indicators of the sources of *n*-alkanes where concentrations are generally measured by conventional GC/MS.

When these hydrocarbons are measured from within a complex mixture using conventional GC/MS, large distinct peaks are observed above a rising baseline (or UCM). Although the retention times of *n*-alkanes are very different from one another, their retention times may be similar to other structural isomers, particularly for larger carbon numbers, owing to the increased probability of possible isomers. If co-elution occurs, however, then the MS used in conjunction with GC could potentially distinguish between the two co-eluting peaks, unless they have similar mass spectra at 70eV.

Figure 5A and 5B shows the contour plots of two portions of the motor oils, focusing on the *n*-alkanes between C₁₈ and C₂₂ (see Figure S1 for contour plot for 5W30 for *n*-alkanes C₂₃ to C₃₀). Figure 5A (synthetic 5W30) shows the presence of an unknown species (compound X) eluting at the same retention time as the *n*-alkane (in the X-axis), and is separated by polarity in the Y-axis. The MS of compound X (see Figure 5A) possesses the molecular ion of the C_n *n*-alkane + CH₂ (*m/z* 14) at 14eV, suggesting that it is a branched alkane belonging to the C_{n+1} aliphatic alkane. This is illustrated by Figure 5A where the black lines signify the overlap between the isomers of two *n*-alkanes. The mass spectrum of compound X at 70eV, however, cannot be used to distinguish between the C_n *n*-alkane and the C_{n+1}-alkane isomer, as there is no molecular ion (particularly for the larger *n*-alkanes, >C₁₉), see Figure 5(A). This unknown species is not distinguishable from the *n*-alkane (for >C₁₉) when analysing using conventional GC (or by GC×GC with shorter secondary columns), as the retention time (volatility axis) of compound X is that of the *n*-alkane and therefore it will co-elute. This co-elution cannot be separated by its MS at 70eV as the primary and secondary ions that would be used to identify both compound X and the *n*-alkane would be the same (*i.e.* *m/z* 43, 57, 71, 85) see embedded MS in Figure 5A. In Figure 5A, the volume of compound X co-eluting with the *n*-alkane is approximately 40% of the total volume of the two together. Furthermore, the separation of these two compounds by polarity (y-axis) is enhanced when increasing the length of the secondary column (to 4m), as when using a shorter length column, GC Imagev2.3 software was unable to automatically deconvolve the two peaks. This suggests that in studies utilising GC/MS, *n*-alkane concentrations may be overestimated (particularly for >C₁₉) and thus may affect the calculated/reported ratios. According to previously published literature, one possibility of compound X could be a dimethyl isomer belonging to the C_{n+1}-alkane.^{43,60} Dimethyl isomers can be shifted by ~100 delta-Kovats (~1 carbon number),⁴³ whereas tri-methyl and tetramethyl have been reported to be shifted by ~150 and ~200 delta-Kovats.⁶⁰ The shift in delta-Kovats for tetra-methyl alkanes identified in this study for example, 2,6,10,14-tetramethylhexadecane (C₂₀), 2,6,10,14-tetramethylheptadecane (C₂₁), 2,6,10,14-tetramethyloctadecane (C₂₂), 2,6,10,14-

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2 358 tetramethylnonadecane (C_{23} , see Figure 4) etc., are in agreement with previous studies (~2 carbon
3
4 359 numbers)⁶⁰ and therefore are not likely candidates for compound X, due to the presence of the C_{n+1} -
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6 360 alkane molecular ion in the mass spectra of the latter (see Figure 5).
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10 362 *Monocyclic Alkanes and Aromatics*

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12 363 *n*-Alkylcycloalkanes have been recognised as major constituents in sediments and crude oils.⁶¹
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14 364 Worton et al.¹⁷ recently reported that primary organic aerosol (POA) emissions were dominated by
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16 365 branched cycloalkanes with one or more rings and one or more branched alkyl side chain (>80% by
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18 366 mass). The authors investigated the chemical composition of POA and its similarity to lubricating
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20 367 oil. It was shown that cyclic and branched alkanes dominated the mass of POA (>60%), while
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22 368 aromatics and *n*-alkanes accounted for <2 and <5%, respectively. The components of the base oil in
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24 369 this study were predominantly cyclic and branched alkanes (based on TIC signal, see Supporting
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26 370 Information), in agreement with Worton et al.¹⁷, but were not for the 5W30 motor oil, where
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28 371 straight and branched chain alkanes were dominant.¹⁷ This is associated with the differences in
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30 372 physical properties of the oils (*e.g.* viscosity and viscosity index) see Table S1. It has been
31
32 373 suggested that cycloalkanes are predominant compounds found in lubricating oils, owing to their
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34 374 desirable properties making them efficient lubricants across a wide range of operating
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36 375 temperatures.¹⁷
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44 377 *n*-Alkylcyclopentanes and *n*-alkylcyclohexanes were identified by retention times of known
45
46 378 standard materials, mass spectral fragmentation at both ionisation energies and by comparison with
47
48 379 published data.¹⁷ *n*-Alkylcycloheptanes have received considerably less attention, probably due to
49
50 380 the difficulties associated in measuring these compounds with various sensitive detectors or owing
51
52 381 to them being present in trace amounts. Although *n*-alkylcycloheptanes were tentatively identified
53
54 382 they were represented by very small chromatographic peaks in comparison to *n*-alkylcyclohexanes
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56 383 and *n*-alkylcyclopentanes. The selected mass fragment ions for *n*-alkylcyclopentanes, *n*-
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alkylcyclohexanes and *n*-alkylcycloheptanes were m/z 68, m/z 82 and m/z 97 respectively. The identified monocyclic alkanes ranged from C_{12} to C_{26} and their selected ion chromatograms are shown in Figure 6. These could be readily identified by 70 eV ionisation.

Using 14eV fragmentation energy mass spectra, alkyl-methylcyclohexanes (m/z 82+97), alkyl-dimethylcyclohexanes (m/z 82+111) and alkyl-trimethylcyclohexanes (m/z 82+125) were also tentatively identified, (and confirmed by published retention indices for alkyl-methylcyclohexane)⁶² using the summed selected ion chromatogram with their respective mass fragments as is illustrated in Figure 6. The wealth of peaks identified signifies the complexity of the base motor oil with only the monomethyl-alkylcycloalkanes being positively identified. Although it is not possible to determine the positioning of the methyl group on the cyclohexane ring (*i.e.* ortho-, para- or meta-position), it is possible to determine the positioning of branching on the alkyl chain as demonstrated for the monomethyl alkanes. Three $C_{22}H_{44}$ isomers were differentiated by their mass spectral differences (at 14eV), according to the positioning of the methyl group on the alkyl chain (analogous to the monomethylalkanes) and the presence of a methyl group on the cyclohexane ring. All three compounds, 1-methyl-4-pentadecylcyclohexane, (3-methylpentadecyl)cyclohexane and 1-methyl-4-(3-methyltetradecyl)cyclohexane possessed their molecular ion of m/z 308, see Figure 7A – 7C. 1-methyl-4-pentadecylcyclohexane and 1-methyl-4-(3-methyltetradecyl)cyclohexane showed mass fragments of m/z 82+97, signifying a methyl-cyclohexane fragment. A relatively smaller peak at m/z 97 was observed for (3-methylpentadecyl)cyclohexane signifying no methyl group on the cyclohexane ring, and peaks m/z 111, 139, 169 and 197 were relatively large, corresponding to the C-C bond breakage on either side of the methyl group on the alkyl chain, leading to mass fragments C_8H_{15} , $C_{10}H_{19}$, $C_{12}H_{25}$ and $C_{14}H_{29}$, respectively, see Figure 7B. For compound 1-methyl-4-pentadecylcyclohexane a relatively large peak at m/z 211 corresponding to the breaking for the alkyl chain from the cyclohexane ring was observed, as well as peaks at m/z 111, 197, which most likely signify a C_8H_{15} ring fragment and a $C_{14}H_{29}$ aliphatic fragment, see Figure 7A. The compound 1-

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2 410 methyl-4-(3-methyltetradecyl)cyclohexane, however, possessed relatively large peaks at m/z 125,
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4 411 153, 155 and 183 which were significantly different to those of the other two isomers. These peaks
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6 412 correspond to mass fragments C_9H_{17} , $C_{11}H_{21}$, $C_{11}H_{23}$ and $C_{13}H_{27}$, respectively, see Figure 7C.
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8 413
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10 414 Not all (methyl-alkyl)-cyclohexanes were positively identifiable in the motor oil, owing to the
11
12 415 intensities of the mass fragments m/z 82 and 97 being relatively large in comparison to the
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14 416 individual bond cleavages along the alkyl chain. However, the methyl-alkylated cyclohexanes are
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16 417 analogous in their positioning in the chromatograph to that of the n -alkanes and monomethylalkanes
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18 418 (with higher polarity).¹⁷ Similarly to the alkyl-cycloalkanes, a series of alkyl-benzenes (m/z 92)
19
20 419 were positively identified ranging from tridecyl-benzene ($C_{19}H_{32}$) to eicosyl-benzene ($C_{26}H_{46}$).
21
22 420 However, surprisingly, no methyl-alkyl-benzenes were observed in either of the lubricating oils.
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24 421 Alkyl-benzenes are often used as refrigeration lubricants and although they are present in mineral
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26 422 oils, are found in small quantities. This is in agreement with the observed relative intensities of this
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28 423 compound class in the chromatography, with respect to the cycloalkane derivatives which are more
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30 424 abundant.¹⁷
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37 426 *Bicyclic Alkanes*

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39 427 Bicyclic aliphatics occupy the same section of the chromatogram as the monocyclic aliphatics,
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41 428 albeit with higher polarity (larger in the y-axis of the chromatography). Alkyl-
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43 429 decahydronaphthalene (or alkyl-decalin) isomers have received increasing interest as potential
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45 430 petroleum biomarkers. Tran et al.⁶³ tentatively observed this compound class using GC×GC-ToF-
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47 431 MS and stressed that the assignments were necessarily tentative owing to the lack of molecular
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49 432 specificity in the interpretation of the mass spectra. Avila et al.⁶⁴ utilised GC×GC-ToF-MS and EI
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51 433 coupled to Fourier transform cyclotron resonance mass spectrometry to analyse compounds found
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53 434 in heavy gas oils, tentatively identifying alkyl-decalins. More recently, however, Wang et al.⁶⁵
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55 435 characterised these compounds by synthesizing and investigating their mass spectra using GC×GC-
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ToF-MS, reporting the mass spectra and retention times for decalins ranging from C₁ to C₁₁ in alkyl chain length. Worton et al.¹⁷ reported that bicyclic alkanes with various branched alkyl chains contributed about 30% of the observed mass in POA and lubricating oil. Up to 11 % of the observed mass fraction of diesel fuel has also been attributed to bicyclic alkanes.⁴³ In this study, we identify alkyl-decalins without the use of authentic standards owing to the characteristic mass spectra obtained for these compounds, with the presence of their corresponding molecular ion; the mass fragments of the respective observed isomers are in good agreement with the results published by Wang et al.⁶⁵. Alkyl-decalins were identified by their characteristic mass fragment m/z 137 together with the molecular ion (using 14eV spectra). The two closely eluting peaks correspond to the alkyl chain at the 1- and 2- positions of the decahydronaphthalene structure but cannot be distinguished from one another. No (methyl-alkyl)-decalins, however, were observed in either of the lubricating oils, which is in contrast to other studies.¹⁷

Tricyclic Aromatics and Alkanes

Tricyclic aromatic compounds occupy the bottom half of the chromatographic section of the contour plot illustrated in Figure 1B. In the base oil, these compounds were more abundant (according to TIC signal, see Supporting Information) than the bicyclic aromatics/alicyclics but less than the monocyclic aromatics/alicyclics. Selecting mass fragment m/z 191, two groups of tricyclic aromatic compounds were identified; alkyl-anthracenes (or alkyl-phenanthrenes) and alkyl-perhydroanthracenes (or alkyl-perhydrophenanthrenes). The mass spectra of alkyl-anthracenes showed little fragmentation, possessing a dominant molecular ion (M^+) and the m/z 191, signifying the 3 fused benzene rings; whereas the observed mass fragments for alkyl-perhydroanthracenes were m/z 55, 67, 81, 95, 109, 135 with the two most intensive peaks belonging to the M^+ molecular ion and m/z 191. These mass spectra were confirmed using the interpretation of Kiselev et al.⁶⁶, who investigated the mass spectrometry of the stereoisomers of perhydroanthracene and perhydrophenanthrene. Compounds from both tricyclic groups ranged from C₁ (methyl-) to C₁₂

(dodecyl-) in alkyl chain length, corresponding to molecular masses (M^+) of m/z 192 to m/z 360, respectively. Very limited tricyclic aliphatics were found in either of the lubricating oils in contrast with other studies.^{17,67} We attribute this to the differences in formulation of different oils, or to the different methods used as highlighted in a recent study.⁶⁸

CONCLUSION

The primary reason why very little quantitative analysis has been conducted on hydrocarbons of greater than C_{10} is that as a consequence of their huge diversity conventional GC is unable to provide a separation and these compounds appear in the chromatogram as a large hump referred to as UCM. The advent of two dimensional gas chromatography techniques in recent years has provided a means to disaggregate the UCM hump providing the separation of thousands of compounds which can be characterised on the basis of their mass spectra. This however has its limitations as each organic compound, particularly in petroleum or environmental samples deriving from petroleum, has a large number of isomers with rather similar mass spectra. The 70eV mass spectra show minor differences in the fragmentation patterns which offer opportunities for distinguishing isomeric species; however, a substantial limitation is that the mass spectra rarely contain a signal from the molecular ion hence greatly complicating the identification process as rather similar mass-spectra at 70eV can result from compounds of differing carbon chain length, as demonstrated by Figure 4(A). Using a variable energy EI source operating at 14 eV has allowed the generation of mass-spectra with clearly visible molecular ions. The combination of retention times in two dimensions and mass-spectra at low and high ionisation energies confers unparalleled power to identify specific isomers within the chromatograms.

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492 **Conflict of Interest Disclosure**

493 The authors declare no competing financial interest.

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FIGURES

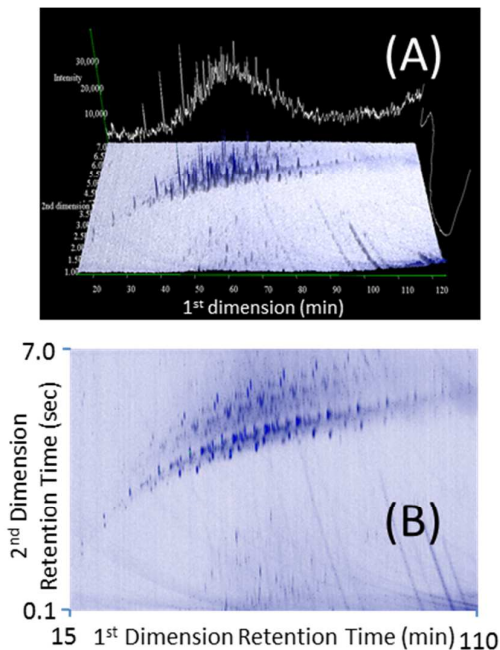


Figure 1. (A) Total ion current chromatogram of a base lubricating oil examined by GC×GC-ToF-MS illustrating improved chromatographic resolution by GC×GC compared with GC (white line in background). **(B)** Contour plot of total ion current chromatogram of a base lubricating oil examined by GC×GC-ToF-MS. Colours indicate the intensities of response (dark blue > blue > white).

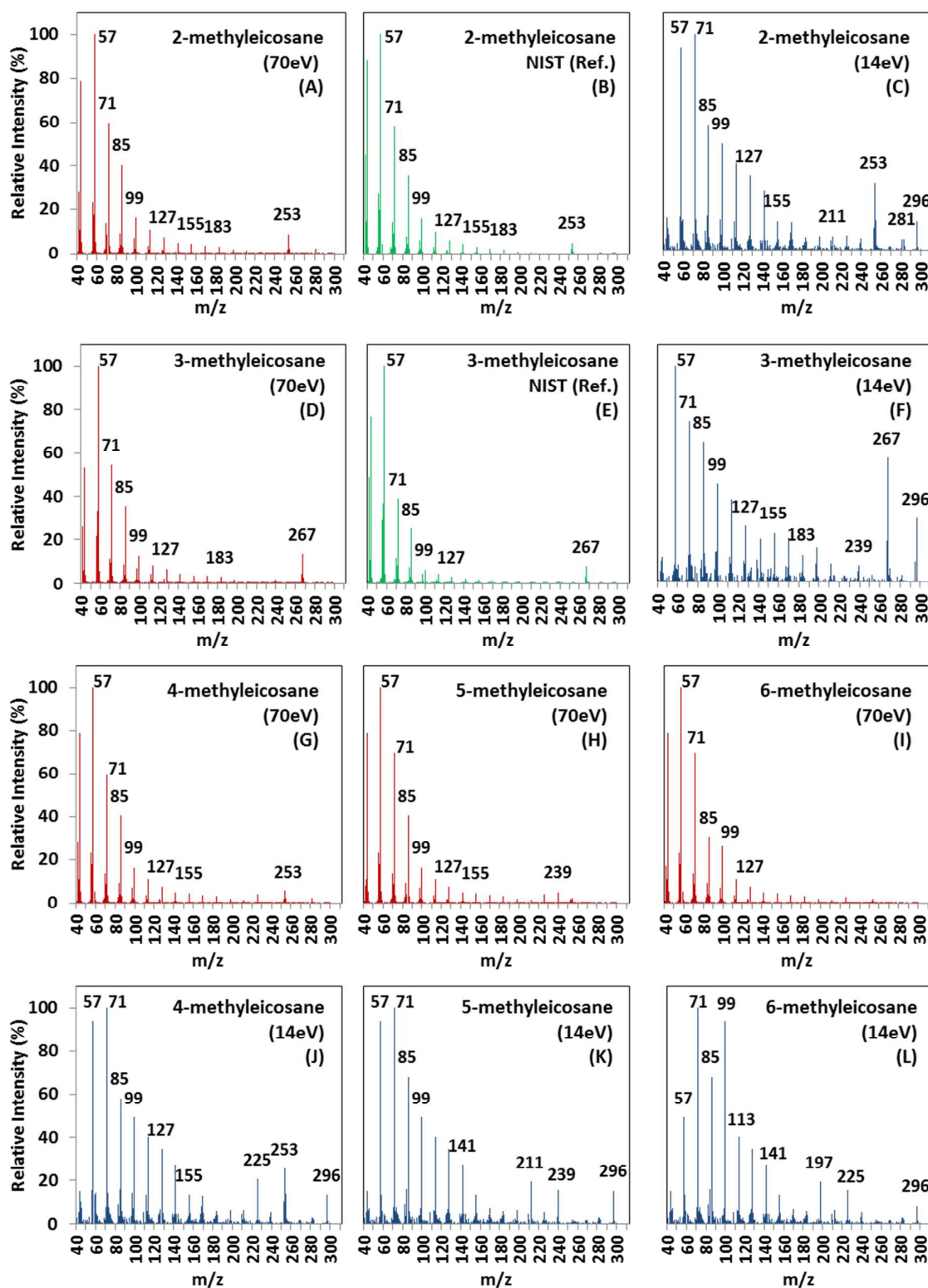


Figure 2. Experimentally determined mass spectra of 2-methyleicosane (at 14eV, 70eV and NIST reference), 3-methyleicosane (at 14eV, 70eV and NIST reference) and 4-, 5- and 6-methyleicosanes (at 14eV and 70eV)

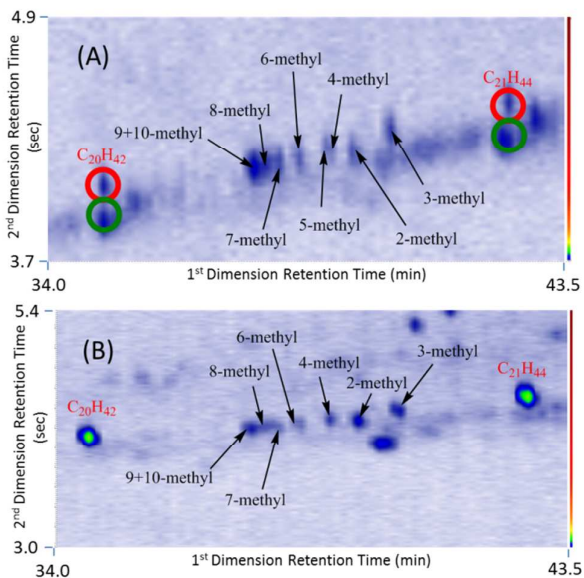


Figure 3. Contour plots of (A) 5W30 lubricating oil; and (B) mineral base oil; illustrating species identified within two *n*-alkane species ($C_{20} - C_{21}$).

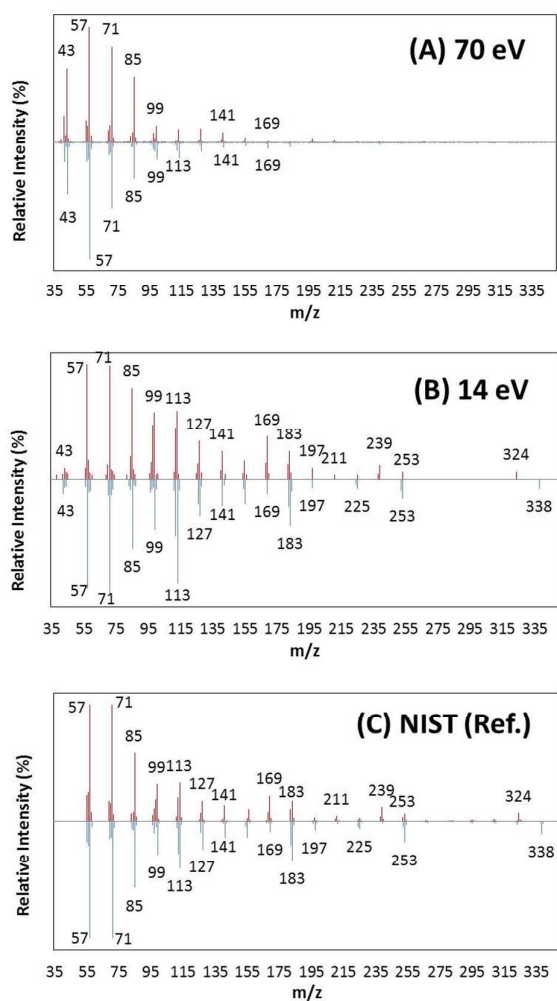


Figure 4. (A) Experimentally determined mass spectra from base lubricating oil for (A) 2,6,10,14-tetramethyl-nonadecane (upper panel) and 2,6,10,14,18-pentamethyl-nonadecane (lower panel) at 70 eV; (B) 2,6,10,14-tetramethyl-nonadecane (upper panel) and 2,6,10,14,18-pentamethyl-nonadecane (lower panel) at 14 eV; and (C) reference spectra of 2,6,10,14-tetramethyl-nonadecane (upper panel) and 2,6,10,14,18-pentamethyl-nonadecane (lower panel) from NIST library.

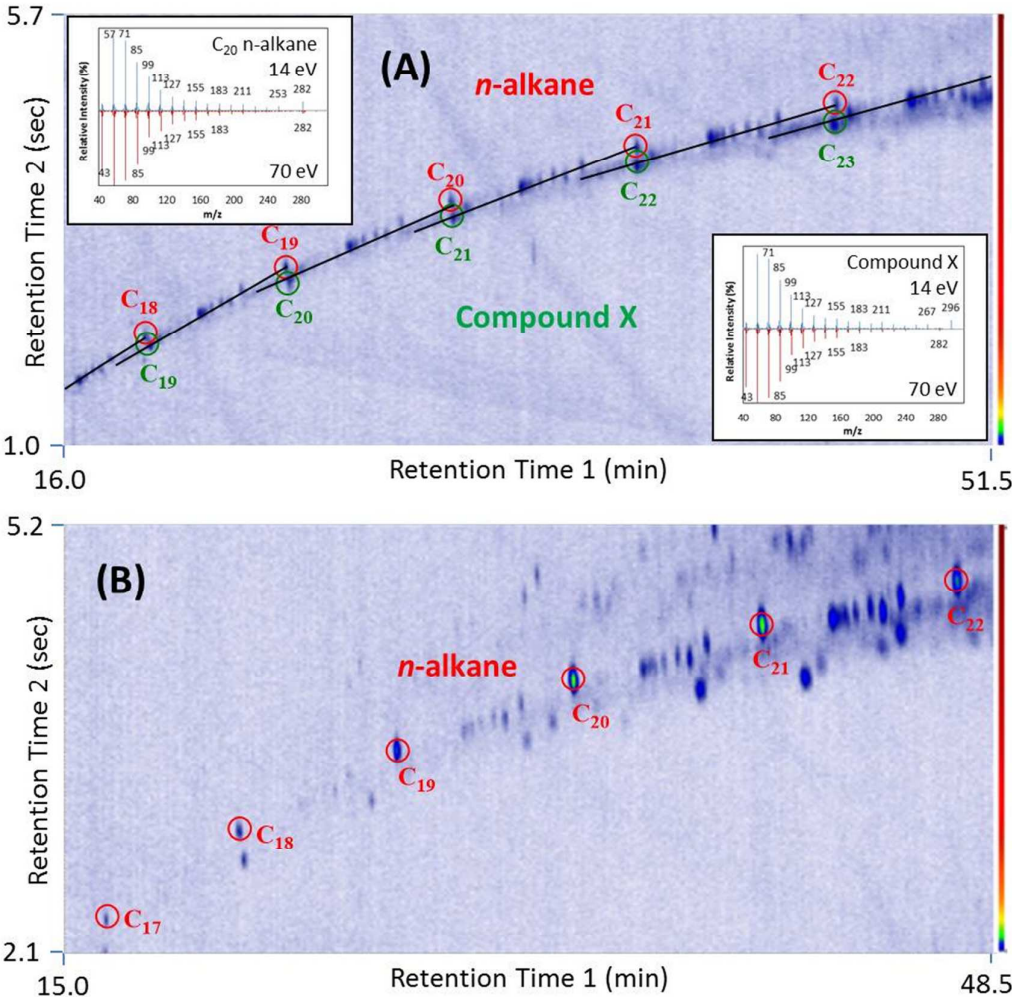


Figure 5. (A) Contour plot of synthetic motor oil 5W30 focusing on C₁₈ to C₂₂. Red circles indicate *n*-alkanes, green circles indicate co-eluting species belonging to the isomer of the (*n*+1)-alkane. Embedded mass spectra of C₂₀ *n*-alkane at 14eV and 70eV in top left and compound X in the bottom right, **(B)** Contour plot of mineral base oil signifying no co-eluting interfering species.

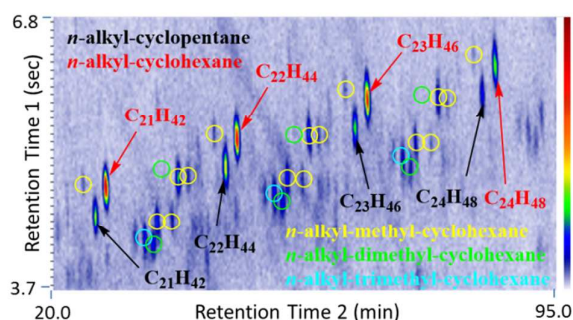


Figure 6. Selected ion chromatogram for summed mass fragments m/z 68+82+97+111, illustrating peaks identified for *n*-alkylcyclopentanes m/z 68 (black font); *n*-alkylcyclohexane m/z 82 (red font); alkyl-methyl-cyclohexanes m/z 82+97 (yellow circles); alkyl-dimethyl-cyclohexanes m/z 82+111 (green circles); and alkyl-trimethyl-cyclohexanes m/z 82+125 (light blue circles).

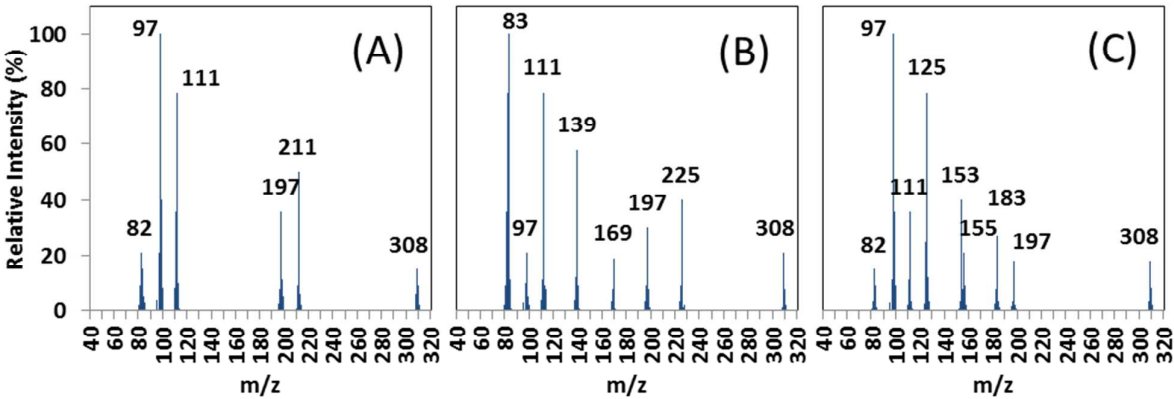
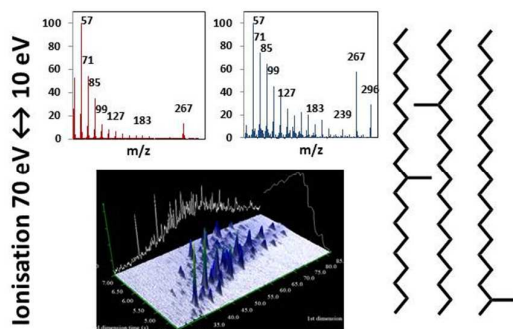


Figure 7. 14eV ionisation energy mass spectra of (A) 1-methyl-4-pentadecylcyclohexane, (B) (3-methylpentadecyl)cyclohexane, and (C) 1-methyl-4-(3-methyltetradecyl)cyclohexane

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